Response to Smallpox Vaccine in Persons Immunized in the Distant Past

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PUBLIC HEALTH STRATEGIES TO thwart potential bioterrorism threats using smallpox are needed. In a previous study, vaccinia-naive participants given undiluted smallpox vaccine (10^8 pfu/mL of the New York City Board of Health strain) were successfully vaccinated with rates of 97.2%. Participants who were given vaccine diluted to a ratio of 1:5 (10^7.2 pfu/mL) had successful vaccination rates of 99.1%, and participants given vaccine diluted to a ratio of 1:10 (10^7.0 pfu/mL) had successful vaccination rates of 97.1%. A 1970 article by Lane et al reported a rate of success with revaccination in 135 (96%) of 161 adults when undiluted vaccine titered at 10^8 pfu/mL and a single pointed needle was used. Significantly more successful revaccination occurs after longer intervals since previous vaccination. Because smallpox vaccine has not been widely used in the United States since 1972, it was anticipated that many previously vaccinated persons would exhibit a major reaction to the smallpox vaccine.

The purpose of this study was to characterize a strategy of vaccination against smallpox with undiluted vaccine or a reduced dose (dilution of 1:3.2, 1:10, or 1:32), followed by revaccination with the same dose if required, in volunteers aged 32 to 60 years with a history of previous smallpox vaccination in the distant past. The data yielded will guide design of a larger trial to more precisely define successful vaccination rates and adverse events in previously vaccinated (non-naive) participants.

METHODS

The study was a randomized, single-blind trial, which was conducted at the National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation Unit at Saint Louis University, St Louis, Mo, and the Institute for Biodefense and Infectious Disease Research, University of Texas Medical Branch, Galveston, Tex. The study was approved by the National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation Unit at Saint Louis University, St Louis, Mo.

Eighty non-naive participants, aged 32 to 60 years, were randomized in a single-blinded study to receive either undiluted or diluted (1:3.2, 1:10, or 1:32) doses of smallpox vaccine. A comparison group, aged 18 to 31 years, of 10 vaccinia-naive participants received undiluted vaccine. Participants were enrolled between April 1 and May 15, 2002, at the National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation Unit at Saint Louis University, St Louis, Mo.

Vaccination of previously vaccinated persons can be successfully revaccinated with a one-fifth (1:5) of the New York City Board of Health strain. Fewer adverse reactions were observed in this study of non-naive participants compared with events in vaccinia-naive participants, which may be due to immunologic memory.

Context There is renewed interest in use of smallpox vaccine due to the potential for a bioterrorist attack. This would involve vaccinating health care workers who were previously vaccinated.

Objective To evaluate the use of diluted vaccinia virus in vaccination of previously vaccinated (non-naive) participants.

Design, Setting, and Participants Eighty non-naive participants, aged 32 to 60 years, were randomized in a single-blinded study to receive either undiluted or diluted (1:3.2, 1:10, or 1:32) doses of smallpox vaccine. A comparison group, aged 18 to 31 years, of 10 vaccinia-naive participants received undiluted vaccine. Participants were enrolled between April 1 and May 15, 2002, at the National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation Unit at Saint Louis University, St Louis, Mo.

Intervention Smallpox vaccine was administered by scarification using 15 skin punctures in the deltoid region of the arm.

Main Outcome Measures Presence of a major reaction, defined as a vesicular or purulent lesion or area of palpable induration surrounding a central lesion following vaccination, and measures of viral shedding and antibody titers.

Results Initial vaccination resulted in a major reaction in 64 of 80 non-naive participants. Ninety-five percent of non-naive participants had major reactions in the undiluted group, 90% in the 1:3.2 dilution group, 81% in the 1:10 dilution group, and 52.6% in the 1:32 dilution group. All (n=10) of the vaccinia-naive participants had major reactions. Compared with vaccinia-naive participants, non-naive participants had significantly smaller skin lesions (P=.04) and significantly less incidence of fever (P=.02). Preexisting antibody was present in 76 of 80 non-naive participants. Antibody responses were significantly higher and occurred more rapidly in the non-naive participants compared with the vaccinia-naive participants (P=.002 for day 28 and P=.003 for 6 months). Vaccinia-naive participants shed virus from the vaccination site 2 to 6 days longer and had significantly higher peak mean viral titers when compared with the non-naive participants (P=.002).

Conclusions Previously vaccinated persons can be successfully revaccinated with diluted (≤1:10) smallpox vaccine. Fewer adverse reactions were observed in this study of non-naive participants compared with events in vaccinia-naive participants, which may be due to immunologic memory.

JAMA. 2003;289:3295-3299

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the site’s institutional review board. Participants were enrolled between April 1 and May 15, 2002, after providing written informed consent.

Healthy adult volunteers between the ages of 32 and 60 years were eligible to participate if they had a smallpox vaccination prior to 1971 and had a typical vaccinia scar or documentation of vaccination. The comparison group consisted of individuals who were otherwise healthy and between the ages of 18 and 31 years, who had never received a smallpox vaccination (vaccinia-naïve). This group received undiluted vaccine to compare maximal expected clinical and immunologic responses.

Individuals were excluded if they had known allergies to any component of vaccine, vaccinia immunoglobulin, cidofovir, or probenecid, or had met other previously reported exclusion criteria.2,5

The primary end point was the rate of major reaction (vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion which may be a crust or ulcer). This reaction indicates that viral multiplication has most likely taken place and that the revaccination is successful.2 Success was defined at 6 to 8 days after intradermal inoculation with a bifurcated needle.

Samples for virus quantitation were obtained from each participant with a vaccinia lesion by rolling a dry swab over the lesion once every 3 to 5 days until the lesion scabbed and was dry. Samples were kept frozen in a reconstituting antibiotic broth until titers were measured by plaque assay as described.8 These tests were performed on the initial vaccination only.

The kinetics of antibody response to vaccinia was determined. A modified neutralization assay was performed on serum samples collected prior to vaccination and between 3 and 5 days after vaccination and 6 and 8 days, 12 and 15 days, 26 and 30 days, and then 6 months postvaccination. Vaccinia-specific binding antibody was determined for the same serum samples by IgG enzymelinked immunosorbent assay. The enzyme-linked immunosorbent assay procedure, modified from the method by Acono-Connors et al,10 included 2-hour incubations at 37°C with the standard washing procedure between the horse-radish peroxidase-conjugated antihuman IgG (Accurate Chemical, Westbury, NY) and the peroxidase substrate (ABTS; Kirkegaard and Perry, Gaithersburg, Md) steps of the procedure. Following a 30-minute incubation at room temperature, a stopping solution (1% sodium dodecyl sulfate) was added to the plates and then the plates were read at a dual wavelength of 405/492 nm. Linear regression plots were determined for each sample tested and end-point titers were determined based on a once daily cut-off of 0.30 nm.

The 95% confidence intervals for the vaccination success rates in each group were obtained using exact methods. Logistic regression modeling was used to estimate the dose effect on the success rate among the previously vaccinated participants. One-sided Fisher exact test

Table 1. Rate of Success of Initial and Repeated Smallpox Vaccination

<table>
<thead>
<tr>
<th>Vaccine Dilution</th>
<th>Titer*</th>
<th>No. of Participants</th>
<th>% (95% CI)</th>
<th>No. of Participants</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>10^5 pfu/mL</td>
<td>10</td>
<td>85.0 (69-97)</td>
<td>10</td>
<td>90.0 (75-100)</td>
</tr>
<tr>
<td>Dilution ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:32</td>
<td>10^3 pfu/mL</td>
<td>10</td>
<td>68.4 (48-87)</td>
<td>10</td>
<td>95.0 (75-100)</td>
</tr>
<tr>
<td>1:10</td>
<td>10^1 pfu/mL</td>
<td>10</td>
<td>90.0 (68-99)</td>
<td>10</td>
<td>95.0 (75-100)</td>
</tr>
<tr>
<td>1:3.2</td>
<td>10^1 pfu/mL</td>
<td>10</td>
<td>90.0 (68-99)</td>
<td>10</td>
<td>95.0 (75-100)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; pfu, plaque-forming unit.

*The titer in each group is the geometric mean titer of all vials of vaccine prepared for use in the study.
†A major reaction is a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion which may be a crust or ulcer. This reaction indicates that virus multiplication has most likely taken place and that the revaccination is successful. Success was defined at 6 to 8 days after intradermal inoculation with a bifurcated needle.
‡Number of participants with success after dose 1 or dose 2. All participants without successful vaccination after dose 1 were revaccinated with the same dose on day 7 or 8.

56 participants had skin preparation with 100% acetone. Overall, the successful vaccination rate difference was 9.2% with 100% acetone having the larger value. The vaccination site was covered with gauze and a semipermeable adhesive dressing. The dressings were changed every 3 to 5 days until the lesion scabbed sufficiently. Participants not manifesting a major reaction after the first vaccination were revaccinated with the same dilution 7 or 8 days after the initial vaccination in an attempt to optimize the rate of major reactions following revaccination.

The smallpox vaccine (New York City Board of Health strain, Dryvax, Wyeth Laboratories, Marietta, Pa; lot number 4008284) was provided by the Centers for Disease Control and Prevention. The vaccine is part of the remaining stock of the vaccine used during the smallpox eradication campaign. All current lots were manufactured between 1974 and 1981. Reformulated vaccine diluent (lot number 1468-1A), which was produced by Chesapeake Biological Laboratories in Baltimore, Md, contained 50% glycerin and 0.21% phenol in sterile water. Unlike the original diluent, the reformulated vaccine did not contain brilliant green dye.

Undiluted (1:3.2) and diluted (1:10) vaccine was prepared by reconstituting vaccinia directly in the vials, which was stored at 2°C to 8°C for up to 56 days after reconstitution. The 1:32 dilution was prepared fresh on each day by diluting 10-fold of the stored 1:3.2 preparation. An aliquot from each vial of vaccine was back-titrated to verify the vaccinia plaque-forming units per milliliter contained in each dilution.

The kinetics of antibody response to vaccinia was determined. A modified neutralization assay was performed on serum samples collected prior to vaccination and between 3 and 5 days after vaccination, 6 and 8 days, 12 and 15 days, 26 and 30 days, and then 6 months postvaccination. Vaccinia-specific binding antibody was determined for the same serum samples by IgG enzymelinked immunosorbent assay. The enzyme-linked immunosorbent assay procedure, modified from the method by Acono-Connors et al, included 2-hour incubations at 37°C with the standard washing procedure between the horse-radish peroxidase-conjugated antihuman IgG (Accurate Chemical, Westbury, NY) and the peroxidase substrate (ABTS; Kirkegaard and Perry, Gaithersburg, Md) steps of the procedure. Following a 30-minute incubation at room temperature, a stopping solution (1% sodium dodecyl sulfate) was added to the plates and then the plates were read at a dual wavelength of 405/492 nm. Linear regression plots were determined for each sample tested and end-point titers were determined based on a once daily cut-off of 0.30 nm.

The 95% confidence intervals for the vaccination success rates in each group were obtained using exact methods. Logistic regression modeling was used to estimate the dose effect on the success rate among the previously vaccinated participants. One-sided Fisher exact test.
was used to assess the difference in the proportion of non-naive responders, between the participants receiving undiluted vaccine and each of the 3 dilutions, and the vaccinia-naive group.

Maximum severity of reactogenicity and local symptoms within 15 days postvaccination were assessed. The results from the non-naive participants were compared with those from the vaccinia-naive participants using the Kruskal-Wallis test for continuous outcomes and the exact or asymptotic contingency table test for categorical outcomes. The generalized estimating equation model or test were used to compare peak antibody titers between the non-naive (undiluted group and all groups combined) with the undiluted vaccinia-naive group.

RESULTS

Ninety participants were vaccinated. Eighty non-naive participants were randomized to receive either undiluted vaccine (n = 20) or a 1:3.2 (n = 20), 1:10 (n = 21), or 1:32 (n = 19) dilution of vaccine (Table 1). Ten vaccinia-naive participants were vaccinated with undiluted vaccine. Eighty-seven (97%) of the participants were white and 53 (59%) were women. The mean (SD) age was 48 (7) years for the non-naive participants and 24 (4) years for the vaccinia-naive participants. Eight of the vaccinia-naive participants were white and 6 were women. There were no statistically significant differences (P < 0.05 by Fisher exact test) in these characteristics among the 4 non-naive groups.

The initial vaccination was successful (Figure 1) in 64 of 80 non-naive participants (Table 1). Ninety-five percent of the participants had a major reaction in the undiluted group, 90% in the 1:3.2 dilution group, 81% in the 1:10 group, and 1:32 in the 52.6% group. All 10 vaccinia-naive participants had a major reaction after initial vaccination. All 16 participants without a major reaction were revaccinated. One of 3 participants in the group receiving the 1:10 dilution had a major reaction on revaccination, which increased the success rate to 85.7%. The generalized estimating equation model or test were used to compare peak antibody titers between the non-naive (undiluted group and all groups combined) with the undiluted vaccinia-naive group.

The local signs and symptoms of vaccinia virus replication among all participants in whom the initial vaccination was successful are summarized in Table 2. The mean (SDs) of the maximum sizes in millimeters of the lesions within 15 days after the initial vaccination among non-naive participants with a major reaction were significantly smaller than those observed among the vaccinia-naive participants (P = 0.04). Although not statistically significant, the mean of the maximum sizes of erythema and induration were smaller in the non-naive group. Maximum mean erythema and maximum mean induration around the vaccination site occurred earlier (between days 6 and 8) in the non-naive participants compared with the vaccinia-naive participants (between days 9 and 11).

Systemic signs and symptoms of adverse events to vaccination were compared between the 64 non-naive participants and the 10 vaccinia-naive participants with vaccinia skin lesions. Fever was significantly less common among the non-naive participants (6 of 64) compared with vaccinia-naive participants (6 of 10; P = 0.02). Other signs and symptoms of adverse events were not significantly different, but the small sample size of the non-naive participants had limited

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Virus Shedding and Antibody Responses

Vaccinia virus was recovered from 246 (67%) of the 368 lesion swab samples collected from participants after initial vaccination between visit 3 (days 3-5) and visit 7 (days 26-30) or until the lesion scabbed. Sixty of 64 non-naive and all 10 vaccinia-naive participants had virus isolated on at least 1 occasion. Vaccinia-naive participants exhibited higher peak mean (SD) titers of virus (3.3 [0.8] log_{10} pfu/mL) than the non-naive participants (P = .002; t test of combined peak mean titers of the naive and non-naive participants). Among the non-naive participants, the peak mean (SD) titers of individuals shedding virus on any day were 3.6 (1.5) pfu/mL for those given undiluted vaccine, 4.0 (1.3) pfu/mL for the participants given 1:3.2 diluted vaccine, 3.4 (1.4) pfu/mL for the participants given 1:10 diluted vaccine, and 4.0 (1.9) pfu/mL for those given 1:32 diluted vaccine. Specifically, the peak mean (SD) titers of virus were 3.0 (1.9) log_{10} pfu/mL for the non-naive participants given undiluted vaccine on day 10; 3.1 (1.9) log_{10} pfu/mL for those given a 1:3.2 dilution of vaccine on day 10; 2.7 (1.5) log_{10} pfu/mL for those given 1:10 on day 14; and 4.4 (1.9) log_{10} pfu/mL for those given 1:32 on day 14 (FIGURE 2). The vaccinia-naive group shed a peak mean (SD) of 4.6 (1.4) log_{10} pfu/mL on day 14.

A clinical response to vaccination with a major reaction resulted in 4-fold antibody or greater increase in 62 (91%) of 68 non-naive participants with a successful vaccination after dose 1 or dose 2. Sixty-seven (99%) of 68 non-naive participants had a 2-fold or greater increase in antibody. Among the 12 non-naive participants without successful vaccination after either dose, 3 had 4-fold or greater increase in neutralizing antibody responses. All vaccinia-naive participants had a 4-fold or greater increase in antibody responses. The kinetics of the serum antibody responses are shown in FIGURE 3A and Figure 3B. Of the 80 non-naive participants, 76 (95%) had preexisting antibody and all vaccinia-naive participants were seronegative before vaccination (P < .001; t test between the vaccinia-naive group and the non-naive groups combined). The geometric mean serum neutralizing antibody titer for prevaccination and postvaccination for days 12-15, 26-30, and 6 months postvaccination was not significantly different among the 4 groups of non-naive participants who manifested major reactions after the initial vaccination. Serum neutralizing antibody responses increased sharply and peaked by days 12-15 in the non-naive participants while the peak antibody response in the vaccinia-naive group did not occur until day 28. Postvaccine antibody titers in the vaccinia-naive participants was significantly lower (P = .002 for day 28 and P = .003 for 6 months) than in the non-naive participants. Binding antibody (enzyme-linked immunosorbent assay) responses (Figure 3B) were similar to the neutralizing antibody results.
The New York City Board of Health strain is the only vaccine approved for use in the United States to prevent smallpox. This study suggests that this vaccine can be diluted to a titer of $10^{0.0}$ pfu/mL (1:3.2 dilution) and still result in a major reaction and antibody production in 90% of previously vaccinated (non-naive) persons. We also found that the vaccine can be diluted to a titer of $10^{0.5}$ pfu/mL (1:10 dilution) and still result in a major reaction and antibody production in 81% to 86% of previously vaccinated (non-naive) participants. These results suggest that it is possible to use the same vaccine dilution in vaccinia-naive and non-naive individuals.

The viral shedding pattern of participants given the 1:32 dilution of vaccine more closely resembled the pattern of vaccinia-naive participants than other non-naive groups. Only 10 of 19 participants given the 1:32 dilution of vaccine developed a primary reaction and those individuals had a similar shedding pattern as the vaccinia-naive participants. This suggests that a subset of non-naive participants was more susceptible to a small vaccine inoculum and the resulting growth curve in this more susceptible subset was similar to that of vaccinia-naive participants.

Preexisting antibody was present in 76 of 80 non-naive participants and preexisting immunity appeared to modify the lesion size, reduced the quantity of virus shed, and reduced adverse events. Vaccination of non-naive participants was associated with smaller skin lesions at the vaccination site and significantly lower incidence of fever than among vaccinia-naive participants. Compared with historical data from our previous study in 665 vaccinia-naive participants, non-naive participants in this study had fewer headaches, chills, malaise, and other clinical events following smallpox vaccination, probably due to immunologic memory. These observations support the US Advisory Committee on Immunization Practices’ recommendation of preferential vaccination of non-naive health care workers when it is feasible.

The vaccination dose did not result in a significant difference in neutralizing antibody mean titer or binding antibody mean titer responses among previously vaccinated participants at any time point. Antibody titers peaked 2 weeks earlier and were significantly higher at all measured time points in non-naive participants compared with the vaccinia-naive participants. The preexisting neutralizing antibodies, early peak in antibody titers, and higher mean titers of antibody in non-naive participants supports the hypothesis that B cell memory is long-lived after smallpox vaccination.

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Acquisition of data: Frey, Newman, Yan, Belshe.
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Critical revision of the manuscript for important intellectual content: Frey, Newman, Yan, Belshe.
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Obtained funding: Belshe.
Administrative, technical, or material support: Frey, Newman, Belshe.
Study supervision: Frey, Newman.
Funding/Support: The research for this study was funded by grant N01-AI-45150 from the National Institute of Allergy and Infectious Diseases.

Acknowledgment: We are indebted to the volunteers who selflessly participated in this study. We are also indebted to Wendy Fanaroff-Ravik, Holli Hamilton, Walla Dempsey, and Stephen Heyse at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Md, and to John Becher and Lisa Roz at the Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, Ga, for their assistance and thoughtful discussions; and to the staff at the Saint Louis University Vaccine and Treatment Evaluation Unit.

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